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DATA EVALUATION RECORD

INDAZIFLAM (BCS-AA10717)

Study Type: OPPTS 870.4200b [§83-2b]; Carcinogenicity Study in Mice

Work Assignment No. 6-1-223 A (MRID 47743416)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Dietary carcinogenicity study in mice; OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 080018 **DP BARCODE:** D292824
TXR #: 0055176

TEST MATERIAL (PURITY): Indaziflam (93.14% a.i.)

SYNONYMS: BCS-AA10717; AE 1170437; *N*-[(1*R*,2*S*)-2,3-dihydro-2,6-dimethyl-1*H*-inden-1-yl]-6-(1-fluoroethyl)-1,3,5-triazine-2,4-diamine

CITATION: Kennel, P. (2008) BCS-AA10717: Carcinogenicity study in the C57BL/6J mouse by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No: SA 05252, December 11, 2008. MRID 47743416. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 47743416), Indaziflam (BCS-AA10717; 93.14% a.i.; Lot/Batch No. EFIM000511) was administered in the diet to C57BL/6J mice (50/sex/dose) for up to 78 weeks at doses of 0, 50, 250, or 1000 ppm (equivalent to 0, 6.8/8.4, 34/42, and 142/168 mg/kg bw/day in males/females). Additionally, groups of 10 mice/sex/dose were treated similarly and sacrificed following 52 weeks of treatment.

No adverse, treatment-related effects were observed on mortality or hematology.

At 1000 ppm, general systemic toxicity was indicated. An increased incidence of wasted appearance was noted in the 1000 ppm females (18.3%) compared to controls (1.7%), first observed after 2 weeks of treatment. Body weights were decreased by 9-18% in both sexes throughout the study. A body weight loss was noted during the first week in both sexes. Body weight gains were decreased by 43-46% in both sexes during Days 1-92, and overall (Days 1-540) body weight gain was decreased by 23% in males and 36% in females. Decreased food consumption was observed throughout the study in both sexes, and overall (Weeks 1-77) food consumption was decreased by 9-12% in both sexes.

Kidney: In the 1000 ppm males, decreased absolute and relative to body kidney weights were noted at the interim (↓15-27%) and terminal sacrifices (↓19-28%). At the terminal sacrifice,

increased incidences of the following minimal to slight findings were observed in kidney (# affected/50 in treated vs controls): (i) collecting ducts hyperplasia (11 vs 1); (ii) pelvic epithelium hyperplasia (8 vs 0); (iii) papillary necrosis (unilateral: 5 vs 0, bilateral: 3 vs 0, and combined: 8 vs 0); and (iv) intratubular yellow brown material (7 vs 0). Additionally, there was a decreased incidence and severity of minimal to marked corticoepithelial vacuolation (3 vs 49).

Stomach: In the 1000 ppm females, increased incidences were observed of macroscopic black focus(i) in the stomach (5/50 treated vs 0/50 controls) and red focus(i) in the stomach in the (4/50 treated vs 1/50 controls). An increased ($p \leq 0.01$) incidence of minimal to moderate glandular erosion/necrosis in stomach (14/49 treated vs 1/50 controls) was noted microscopically.

Ovary: At 1000 ppm, increased incidences of the following lesions were observed grossly in the ovary: enlarged (6/50 treated vs 1/50 control) and black focus(i) (7/50 treated vs 0/50 controls). An increased incidence of blood-filled cyst(s)/follicle(s) (19/49 treated vs 11/50 controls) was also noted.

Equivocal findings were noted in the liver (prominent lobulation in males and redistribution of vacuolation in both sexes) and spleen (weight in females).

The LOAEL is 1000 ppm (equivalent to 142/168 mg/kg/day in males/females). The LOAEL was based on: increased incidence of wasted appearance in females; decreased body weights, body weight gains, and food consumption in both sexes; and indications of renal toxicity and hepatotoxicity in males, and stomach and ovarian toxicity in females. The NOAEL is 250 ppm (equivalent to 34/42 mg/kg/day in males/females).

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on the findings detailed above.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

COMPLIANCE: Signed and dated GLP Compliance, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indaziflam

Description: Light beige powder

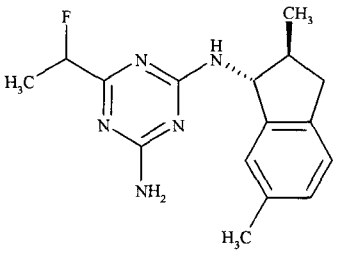
Lot/Batch No.: EFIM000511

Purity (w/w): 93.14% a.i.

Stability of compound: The Sponsor stated that the compound was stable in the diet for up to 92 days at room temperature.

CAS #: 950782-86-2

Structure:



2. Vehicle: Diet

3. Test animals

Species: Mouse

Strain: C57BL/6J

Age and group mean weight at study initiation: Approximately 6 weeks old
20.0 – 20.3 g males; 17.2 – 17.3 g females

Source: Charles River Laboratories (l'Arbresle, formerly St. Germain-sur-l'Arbresle, France)

Housing: Individually. The cages were suspended, stainless steel and wire mesh.

Diet: A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum*, except for overnight fasts prior to sample collection or termination

Water: Filtered and softened tap water, *ad libitum*

Environmental conditions

- **Temperature:** 20-24°C
- **Humidity:** 40-70%
- **Air changes:** 10-15/h
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: 7 days

B. STUDY DESIGN

1. In life dates: Start: 2/8/2006 End: 8/28/2007

2. Animal assignment: Animals were randomly assigned, stratified by body weight, to the test groups presented in Table 1.

TABLE 1: STUDY DESIGN [a]				
Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day; M/F)	Main study 78 weeks (# mice/sex)	Interim kill 52 weeks (# mice/sex)
Control	0	0/0	50	10
Low (LDT)	50	6.8/8.4	50	10
Mid (MDT)	250	34/42	50	10
High (HDT)	1000	142/168	50	10

a Data were obtained from text table on page 22 of MRID 47743416.

3. **Dose-selection rationale:** The dose levels were selected based on the results from a previous 90-day dietary study in the mouse (Study Report # SA 04094), where dietary administration at 1200 ppm (high dose) resulted in one death. Other treatment-related effects at this dose included: (i) decreased body weights and food consumption in both sexes; (ii) decreased mean serum albumin in males; and (iii) decreased total serum protein in females. No effects were noted at 100 or 500 ppm in either sex.

4. **Treatment preparation, analysis, and administration:** The test substance was incorporated into the diet to provide the required dietary concentrations. The test substance formulations were prepared approximately every 8 weeks and were stored at room temperature. A total of 11 formulations for each dose group were prepared.

   The Sponsor stated that stability of the test substance in the diet has been demonstrated in a previous study (SA 04029) where samples of 20 and 10,000 ppm where analyzed after storage either frozen for 80 days followed by 12 days at room temperature or 92 days at room temperature (data not reported in this study report). This time period covered the period of storage and usage for this study. The homogeneity of the test substance in diet was verified at each concentration on the first formulation (F1) and at 50 and 1000 ppm on the formulation F6, to demonstrate adequate formulation procedures. The mean value obtained from the homogeneity check was taken as measured concentration. Additionally, the concentration was checked on formulations F3, F6, and F9 at all concentrations not checked for homogeneity.

Results

Homogeneity (% coefficient of variation): 1.29-3.23

Stability (% of initial): Not reported

Concentration (% of nominal): 90-104%

Dose (ppm)	Range (% of nominal)
50	94-104
250	92-99
1000	90-101

Assuming that the stability data do confirm the test compound's stability in the diet for 8 weeks at room temperature, the analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable. Stability data needs to be submitted.

5. **Statistics:** The following analyses were conducted and significance was denoted at the 5% and 1% levels.

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight gain Terminal body weight Organ weight Hematology [a]	Bartlett's test for homogeneity	One-way analysis of variance (ANOVA) followed with Dunnett's test (2-sided) when significant	Kruskal-Wallis test followed with Dunn's test (2-sided) when significant
Body weight Food consumption Hematology [b]	Bartlett's test for homogeneity [c]	One-way ANOVA on raw data or transformed data followed with Dunnett's test (2-sided) when significant	Kruskal-Wallis test followed with Dunn's test (2-sided) when significant
Survival	Kaplan-Meier estimation procedures were used. Statistical significance of differences in survival rates between treated and control groups and dose related trends in survival was assessed using Cox's and Tarone's tests on life table data. Probability values presented are 2-sided for pairwise comparisons and trend test.		
Non-neoplastic and neoplastic histology [d]	When the incidences of the 50 and 250 ppm treated groups were equal to 0, only the 1000 ppm group was compared to the control group and no trend test was performed. When the number of lesion-bearing animals was equal to 2 in one group and was equal to 0 in the other groups, no statistical analysis was performed. Selected lesions were analyzed by Cochran-Armitage method for trend (1-sided) and the Fisher's exact test (1-sided) for control versus treatment comparisons. Further survival adjusted analyses, considering any possible intercurrent mortality differences due to the competing toxicity among the treated groups, were performed on lesions. Selected incidental tumors and non-neoplastic lesions were analyzed by logistic regression of tumor prevalence. Fatal tumors were analyzed by the life-table test. Statistical significance of differences in incidences between treated and control groups and dose-related trends were investigated using Cox's and Tarone's tests.		

a Hematology parameters included hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, % neutrophils, and % lymphocytes.

b Hematology parameters included red blood cell count, platelet count, white blood cell count, neutrophil count, and lymphocyte count.

c When Bartlett's test was significant, data were transformed using the log transformation for body weight and food consumption parameters or using the square root transformation for hematology parameters. Bartlett's test was then run on the transformed data.

d It was stated that: "There was no microscopic pathology – neoplastic lesion that suggested an increased tumor incidence related to the administration of the test material. Consequently, no statistical analysis was performed on tumors."

These statistical analyses were considered appropriate, assuming that a normal data distribution was confirmed prior to parametric testing.

C. METHODS

1. Observations

a. Cageside observations: Animals were checked for moribundity and mortality twice daily (once daily on weekends or public holidays). All animals were observed for clinical signs at least once daily.

b. Clinical examinations: Detailed physical examinations including palpation for masses were performed at least weekly.

c. Neurological evaluations: As the neurotoxicological potential was examined in other studies (reports not cited), these determinations were not conducted in the present study.

2. Body weight: Each animal was weighed prior to treatment, weekly for the first 13 weeks of study, approximately every 4 weeks thereafter, and prior to necropsy.

3. Food consumption and compound intake: Food consumption (g/mouse/day) was recorded weekly up to Week 13 and once approximately every 4 weeks thereafter. Compound intake (mg/kg/day) was calculated based on nominal concentration, food consumption, and body weight values.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed, but are not a guideline requirement under OPPTS 870.4200b.

5. Hematology: Blood was sampled from Isoflurane anesthetized animals by puncture of the retro-orbital venous plexus after overnight dietary fasting. Blood analyses were performed on all the surviving animals of the 12-month interim sacrifice groups and on the first 10 surviving mice of the terminal sacrifice groups on Weeks 53, 54, or 55. Prior to necropsy, hematology was performed on the first twenty surviving mice of the terminal sacrifice groups on Weeks 79 or 80. When possible, a blood smear was prepared for the moribund animals, just before sacrifice. At sacrifice, blood smears were prepared for all animals. Blood smears were only analyzed when other hematology parameters were abnormal. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (Cont. and HDT unless effects are observed) based on Guideline 870.4200 and OECD 451

Clinical chemistry analyses were not performed; however, clinical chemistry analyses are not a guideline requirement under OPPTS 870.4200b.

6. **Urinalysis:** Urinalysis was not performed and is not a guideline requirement under OPPTS 870.4200b.

7. **Sacrifice and pathology:** On Days 371, 372, or 373 for the interim sacrifice group, and on Days 552 to 567 for the terminal sacrifice group, all surviving animals were sacrificed by exsanguination under deep anesthesia (Isoflurane). An approximately equal number of animals randomly distributed amongst all groups were sampled on each day. Animals were diet fasted overnight prior to sacrifice. All animals, including unscheduled decedent animals, were necropsied. The following organs or tissues were sampled (X) and weighed (XX) at necropsy:

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland [a]
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
		XX	Ovaries*+	X	Harderian gland
	RESPIRATORY	XX	Uterus (with cervix)*+	X	Articular surface (knee joint)
X	Trachea*	X	Mammary gland*	X	All gross lesions and masses*
X	Lung*++	X	Vagina		
X	Nose* [a]				
X	Pharynx* [a]				
X	Larynx* [a]				

a Collected, but not examined
* Recommended for carcinogenicity studies based on Guideline 870.4200
+ Organ weight required in carcinogenicity studies
++ Organ weight required if inhalation route

Samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye and optic nerve, Harderian gland, epididymis, and testis that were fixed in Davidson's fixative. The lacrimal gland, larynx, pharynx, and nasal cavities were not processed but were retained in fixative for possible future examination. Otherwise, the remaining tissues listed above from the terminal sacrifice group were routinely processed, stained with hematoxylin and eosin, and examined microscopically. No histopathological examination was performed for the interim sacrifice group, and histological slides were not prepared for samples from that group. Histopathological examinations were performed on all organs and tissues embedded in all animals from all groups. For all unscheduled sacrificed or dead animals on study, the cause of death was determined when possible.

Following the initial examination by the Study Pathologist, an external review pathologist undertook an independent peer-review of representative slides and diagnoses according to standardized operating procedures. The diagnoses presented in this report represent the consensus opinion of the two pathologists.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity:** An increased incidence of wasted appearance was noted in the 1000 ppm females (18.3%) compared to controls (1.7%). This clinical sign was first observed after 2 or 3 weeks of treatment in five animals and lasted 1 or 2 weeks. Other occurrences were noted later in the study. The incidences of other clinical signs in the treated groups were similar to controls.

2. **Mortality:** Mortality rates in the treated groups were generally similar to controls at both the interim sacrifice (1.7-8.3%) and terminal sacrifice (10.0-26.0%), and a dose-response relationship was not clearly shown.

B. BODY WEIGHT AND BODY WEIGHT GAINS: Decreased ($p \leq 0.01$) body weights were observed throughout the study in the 1000 ppm males (↓9-15%) and females (↓10-18%; Table 2). Minor differences ($p \leq 0.05$) of 3% were noted in the 250 ppm females compared to controls on 3 occasions. Otherwise, body weights in the treated groups were similar to controls.

At 1000 ppm, a body weight loss was noted during the first week in the males (-0.3 g treated vs 1.6 g controls) and females (-0.6 g treated vs 1.4 g controls). Decreased ($p \leq 0.05$) body weight gains were also observed in the males for Days 1-92 (↓43%) and 92-176 (↓10%) and in females for Days 1-92 (↓46%), 176-372 (↓36%), and 372-540 (↓43%). Decreased ($p \leq 0.01$) overall (Days 1-540) body weight gain was noted in the males (↓23%) and females (↓36%). A decreased ($p \leq 0.01$) body weight gain was also observed in the 250 ppm females on Days 1-8 (↓29%); however, body weight gain was similar to controls during Days 1-92 and overall. Other differences ($p \leq 0.05$) in body weight gains were considered minor and transient.

TABLE 2. Mean (±SD) body weights and body weight gains (g) at selected intervals in mice treated with Indaziflam in the diet for up to 540 days. [a]

Days(s)	Dose (ppm)			
	0	50	250	1000
Males				
1	20.3+1.0	20.1+1.1	20.2+1.1	20.0+1.0
288	30.8+1.8	30.3+1.9	30.2+2.2	26.3+1.3** (↓15)
344	31.0+1.8	30.9+1.6	30.6+2.3	27.1+1.5** (↓13)
540	31.0+1.9	31.6+1.7	31.5+2.0	28.2+1.8** (↓9)
BWG (1-8)	1.6+0.6	1.5+0.6	1.7+0.6	-0.3+0.7**
BWG (1-92)	6.7+0.9	6.7+1.3	6.8+1.4	3.8+1.2** (↓43)
BWG (92-176)	2.0+1.4	2.0+1.0	2.2+0.7	1.8+0.7* (↓10)
BWG (176-372)	1.9+0.9	2.0+1.1	1.8+1.4	1.7+0.9
BWG (372-540)	0.0+1.0	0.8+1.1**	0.7+1.2**	0.8+0.8**
BWG (1-540)	10.7+1.5	11.6+1.6* (↑8)	11.4+1.7	8.2+1.5** (↓23)
Females				
1	17.2+0.7	17.2+0.7	17.2+0.8	17.3+0.7
8	18.6+0.9	18.5+0.9	18.2+0.8	16.7+0.7** (↓10)
43	21.6+0.8	21.4+0.9	21.0+0.9* (↓3)	17.8+1.3** (↓18)
344	26.0+1.5	26.5+1.4	25.6+1.6	22.6+1.0** (↓13)
540	27.9+2.1	28.1+1.8	27.2+2.0	24.2+1.0** (↓13)
BWG (1-8)	1.4+0.6	1.4+0.7	1.0+0.6** (↓29)	-0.6+0.5**
BWG (1-92)	5.4+1.1	5.5+0.8	5.4+0.7	2.9+1.4** (↓46)
BWG (92-176)	1.8+0.8	2.1+0.9	1.6+0.6	1.5+0.8
BWG (176-372)	2.2+0.9	2.0+1.1	2.0+1.0	1.4+0.6** (↓36)
BWG (372-540)	1.4+1.1	1.5+1.2	1.0+1.5	0.8+0.6** (↓43)
BWG (1-540)	10.6+1.9	11.0+1.7	9.9+1.9	6.8+1.1** (↓36)

a Data were obtained from text-table on page 34 and Tables 3-4d on pages 71-93 in MRID 47743416. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

* Significantly different ($p \leq 0.05$) from the control groups

** Significantly different ($p \leq 0.01$) from the control groups

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** At 1000 ppm, decreased overall (Weeks 1-77) food consumption was noted in the males (↓9%) and females (↓12%; Table 3). Decreased food consumption was observed throughout the study in both sexes at 1000 ppm. The maximum decrease was 18% compared to controls, and the differences were significant ($p<0.01$) at all time points in both sexes, except males on Day 540. Other differences ($p<0.05$) in food consumption in the treated groups compared to controls were minor and transient.

TABLE 3.[a]

GROUP MEAN FOOD CONSUMPTION (g/animal/day)								
Sex	Males				Females			
Dose level of BCS-AA10717 (ppm)	0	50	250	1000	0	50	250	1000
Weeks 1-13 (% *vs* control)	3.91 -	3.95 (101)	3.97 (102)	3.56 (91)	3.88 -	3.95 (102)	3.89 (100)	3.38 (87)
Weeks 1-53 (% *vs* control)	3.97 -	3.99 (101)	4.02 (101)	3.61 (91)	4.01 -	4.07 (101)	4.02 (100)	3.55 (89)
Weeks 1-77 (% *vs* control)	3.99 -	4.01 (101)	4.02 (101)	3.64 (91)	4.11 -	4.16 (101)	4.09 (100)	3.61 (88)

a Copied from text table on page 35 of MRID 47743416.

2. **Compound consumption:** The mean achieved dosages are reported in Table 1.

D. **HEMATOLOGY:** No adverse, treatment-related effects were observed on hematology parameters. All differences ($p \leq 0.05$) were minor, transient, and/or unrelated to dose.

E. **SACRIFICE AND PATHOLOGY**

1. **Organ weights:** After 1 year of treatment, no adverse, treatment-related effect was observed on organ weights. Decreased ($p \leq 0.01$) absolute and relative to body kidney weights were noted in the 1000 ppm males (↓15-27%). The decreased relative weight was slight, and there were no reported corroborating effects of toxicity; therefore, this effect was considered equivocally adverse.

   After 18 months of treatment, decreased ($p < 0.01$) terminal body weights were noted in the 1000 ppm group (↓11-15%; Table 4). Decreased ($p < 0.01$) absolute and relative to body kidney weights were observed in the 1000 ppm males (↓19-28%), and absolute and relative spleen weights in the 250 and 1000 ppm females (↓38-59%).

TABLE 4. Selected organ weights (g) in mice treated with Indaziflam in the diet for 18 months. [a]

Organ	Dose (ppm)			
	0	50	250	1000
Males				
Terminal body weight (g)	27.1+2.0	27.5+1.8	27.3+2.2	24.0+1.7** (↓11)
Kidneys Absolute (g)	0.538+0.061	0.518+0.049	0.526+0.065	0.387+0.046** (↓28)
Relative to BW (%)	1.985+0.178	1.893+0.148* (↓5)	1.922+0.160	1.611+0.137** (↓19)
Females				
Terminal body weight (g)	24.7+2.1	24.9+1.6	24.1+1.9	20.9+1.2** (↓15)
Spleen Absolute (g)	0.180+0.212	0.151+0.099	0.108+0.025** (↓40)	0.073+0.016** (↓59)
Relative to BW (%)	0.720+0.812	0.606+0.399	0.448+0.091** (↓38)	0.350+0.070** (↓51)

a Data (n=37-42) were obtained from Table 8b on pages 127-132 of MRID 47743416. Percent difference from controls, calculated by reviewers, is included in parentheses.
* Significantly different ($p<0.05$) from the control group
** Significantly different ($p<0.01$) from the control group

Differences ($p<0.05$) in organ weights at the interim and terminal sacrifices that were not discussed were not considered adverse, treatment-related effects. These differences were minor; toxicity was not corroborated by clinical and pathological findings; and/or the findings were considered related to the decreased terminal body weights rather than test compound toxicity.

2. **Gross pathology:** At the interim sacrifice, no treatment-related effect was observed on the incidence of macroscopic lesions.

In animals necropsied at the terminal sacrifice (including decedents), increased incidences of the following lesions were observed grossly (# affected/50 in treated vs control groups; Table 5): (i) prominent lobulation in the liver in 1000 ppm males (15 vs 1); (ii) black focus(i) in the stomach in the 1000 ppm females (5 vs 0); (iii) red focus(i) in the stomach in the 1000 ppm females (4 vs 1); (iv) enlarged ovary at 250 and 1000 ppm (5-6 vs 1); and (v) black focus(i) in the ovary at 250 and 1000 ppm (4-7 vs 0). The incidences of other findings in the treated groups were similar to the control groups or without histological correlate.

TABLE 5. Incidence (# affected/50) of macroscopic lesions in selected organs of mice treated with Indaziflam in the diet for up to 18 months. [a]

Lesion		Dose (ppm)			
		0	50	250	1000
Males					
Liver	Prominent lobulation	1	3	3	15
Females					
Stomach	Black focus(i)	0	1	1	5
	Red focus(i)	1	0	1	4
Ovary	Enlarged	1	3	5	6
	Black focus(i)	0	2	4	7

a Data were obtained from Table 9d on pages 150-155 of MRID 47743416.

3. Microscopic pathology

a. Non-neoplastic: Selected histological findings are presented in Tables 6a and 6b. At the terminal sacrifice, increased ($p \leq 0.05$, except as noted) incidences of the following minimal to slight findings were observed in kidney in the 1000 ppm males (# affected/50 in treated vs controls; severity): (i) collecting ducts hyperplasia (11 vs 1); (ii) pelvic epithelium hyperplasia (8 vs 0); (iii) papillary necrosis (unilateral: 5 vs 0, bilateral: 3 vs 0; NS, and combined: 8 vs 0); and (iv) intratubular yellow brown material (7 vs 0). Additionally, there was a decreased ($p \leq 0.01$) incidence and severity of minimal to marked corticoepithelial vacuolation in the 1000 ppm males (3 vs 49).

At 1000 ppm, increased ($p \leq 0.01$) incidences of diffuse, mainly centrilobular, hepatocellular vacuolation was noted in males (35/50 treated vs 14/50 controls; minimal to moderate) and females (35/50 treated vs 8/50 controls; minimal to marked), and decreased ($p \leq 0.01$) incidences of diffuse hepatocellular vacuolation was observed in males (3/50 treated vs 17/50 controls) and females (5/50 treated vs 33/50 controls).

TABLE 6a. Incidence (# affected/50) of microscopic lesions in selected organs of male mice treated with Indaziflam in the diet for up to 18 months. [a]

Lesion		Dose (ppm)			
		0	50	250	1000
Kidney	Collecting ducts hyperplasia, focal/multifocal (total)	1	0	1	11**
	Minimal	1	0	0	5
	Slight	0	0	1	6
	Pelvic epithelium hyperplasia, focal/multifocal (total)	0	1	2	8**
	Minimal	0	1	2	7
	Slight	0	0	0	1
	Papillary necrosis, unilateral, focal/multifocal (total)	0	0	0	5*
	Minimal	0	0	0	3
	Slight	0	0	0	2
	Papillary necrosis, bilateral, focal/multifocal (total)	0	0	0	3
	Minimal	0	0	0	1
	Slight	0	0	0	2
	Papillary necrosis, combined uni/bilateral (total)	0	0	0	8**
	Intratubular yellow brown material	0	0	1	7**
	Minimal	0	0	1	5
	Slight	0	0	0	2
	Corticoepithelial vacuolation, multifocal/diffuse (total)	49	47	47	3**
	Minimal	1	4	2	3
	Slight	4	8	5	0
	Moderate	29	23	21	0
	Marked	15	12	19	0
Liver	Hepatocellular vacuolation, mainly centrilobular, diffuse (total)	14	13	12	35**
	Minimal	12	12	10	12
	Slight	1	1	2	11
	Moderate	1	0	0	12
	Hepatocellular vacuolation, diffuse (total)	17	22	21	3**
	Minimal	16	18	17	2
	Slight	1	3	3	0
	Moderate	0	1	1	1

a Data were obtained from text tables on pages 41-44 and Table 10c on pages 188-203 of MRID 47743416.

In the 1000 ppm females, increased ($p \leq 0.01$, except as noted) incidences of the following findings were observed (# affected/# examined in treated vs controls; severity): (i) glandular erosion/necrosis in stomach (14/49 vs 1/50; minimal to moderate); (ii) eosinophilic cytoplasmic alteration in gallbladder (13/46 vs 3/49; minimal to moderate); and (iii) blood-

filled cyst(s)/follicle(s) (19/49 vs 11/50; minimal to moderate; statistical analysis results not reported).

The incidence of other microscopic lesions were similar to controls or the increase was minor without corroborating evidence of an adverse response in the organ.

TABLE 6b. Incidence (# affected/50) of microscopic lesions in selected organs of female mice treated with Indaziflam in the diet for up to 18 months. [a]

Lesion		Dose (ppm)			
		0	50	250	1000
Liver	Hepatocellular vacuolation, mainly centrilobular, diffuse (total)	8	9	9	35**
	Minimal	4	1	1	1
	Slight	3	6	6	7
	Moderate	1	2	2	16
	Marked	0	0	0	11
	Hepatocellular vacuolation, diffuse (total)	33	29	34	5**
	Minimal	12	5	11	2
	Slight	10	14	16	1
	Moderate	9	8	5	2
	Marked	2	2	2	0
Stomach	Glandular erosion/necrosis, focal/multifocal (total)	1	2	1	14 [b]**
	Minimal	1	2	1	4
	Slight	0	0	0	9
	Moderate	0	0	0	1
Gallbladder	Eosinophilic cytoplasmic alteration, focal/multifocal (total)	3 [b]	5	3 [b]	13 [c]**
	Minimal	1	0	0	6
	Slight	1	2	1	6
	Moderate	1	2	2	1
	Marked	0	1	0	0
Ovary(ies)	Blood-filled cyst(s)/follicle(s), focal/multifocal (total)	11	10	12 [d]	19 [b]
	Minimal	10	8	4	14
	Slight	1	2	5	4
	Moderate	0	0	3	1

a Data were obtained from text tables on pages 41-43 and Table 10c on pages 188-203 of MRID 47743416.
b 49 animals were examined instead of 50.
c 46 animals were examined instead of 50.
d 48 animals were examined instead of 50.

b. Neoplastic: The incidences of neoplastic lesions in the treated groups were similar to controls.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 1000 ppm, and the NOAEL was 250 ppm. At 1000 ppm, an increased incidence of wasted appearance was noted in the females. Decreased body weight, body weight gain, and food consumption were noted in both sexes. Decreased kidney and liver weights were noted in both sexes. At necropsy, prominent lobulation in the liver was found in 15/40 males, and red or black foci on the stomach were observed in 3/40 males and 8/40 females. Non-neoplastic changes were noted in the kidney, liver, and stomach. In the kidney, a higher incidence of collecting ducts hyperplasia (bilateral), pelvic epithelium hyperplasia (bilateral), unilateral and bilateral papillary necrosis (tip of papilla), and intratubular yellow brown material, and a lower incidence of corticoepithelial vacuolation were noted in males. In the liver, a higher incidence and/or severity of mainly centrilobular hepatocellular vacuolation and a concomitant decreased incidence of diffuse hepatocellular vacuolation were noted in both sexes. In the stomach, a higher incidence of glandular erosion/necrosis was noted in females. No increased incidences of neoplastic lesions were noted.

B. REVIEWER COMMENTS: No adverse, treatment-related effects were observed on mortality or hematology.

At 1000 ppm, general systemic toxicity was indicated. An increased incidence of wasted appearance was noted in the 1000 ppm females (18.3%) compared to controls (1.7%). This clinical sign was first observed after 2 or 3 weeks of treatment in five animals and lasted 1 or 2 weeks. Other occurrences were noted later in the study. Decreased ($p \leq 0.01$) body weights were observed throughout the study in the males (↓9-15%) and females (↓10-18%). A body weight loss was noted during the first week in the males (-0.3 g treated vs 1.6 g controls) and females (-0.6 g treated vs 1.4 g controls). Decreased ($p \leq 0.05$) body weight gains were also observed in the males for Days 1-92 (↓43%) and 92-176 (↓10%) and in females for Days 1-92 (↓46%), 176-372 (↓36%), and 372-540 (↓43%). Decreased ($p \leq 0.01$) overall (Days 1-540) body weight gain was noted in the males (↓23%) and females (↓36%). Decreased food consumption was observed throughout the study in both sexes, and decreased overall (Weeks 1-77) food consumption was noted in the males (↓9%) and females (↓12%). The maximum decrease was 18% compared to controls, and the differences were significant ($p < 0.01$) at all time points in both sexes, except males on Day 540.

Kidney: In the 1000 ppm males, decreased ($p \leq 0.01$) absolute and relative to body kidney weights were noted at the interim sacrifice (↓15-27%) and terminal sacrifice (↓19-28%). At the terminal sacrifice, increased ($p \leq 0.05$, except as noted) incidences of the following minimal to slight findings were observed in kidney (# affected/50 in treated vs controls; severity): (i) collecting ducts hyperplasia (11 vs 1); (ii) pelvic epithelium hyperplasia (8 vs 0); (iii) papillary necrosis (unilateral: 5 vs 0, bilateral: 3 vs 0; NS, and combined: 8 vs 0); and

(iv) intratubular yellow brown material (7 vs 0). Additionally, there was a decreased ($p \leq 0.01$) incidence and severity of minimal to marked corticoepithelial vacuolation (3 vs 49).

Stomach: In the 1000 ppm females, increased incidences were observed of macroscopic black focus(i) in the stomach (5/50 treated vs 0/50 controls) and red focus(i) in the stomach in the (4/50 treated vs 1/50 controls). An increased ($p \leq 0.01$) incidence of minimal to moderate glandular erosion/necrosis in stomach (14/49 treated vs 1/50 controls) was noted microscopically.

Ovary: Increased incidences of the following lesions were observed grossly: enlarged ovary at 250 and 1000 ppm (5-6/50 treated vs 1/50 control) and black focus(i) in the ovary at 250 and 1000 ppm (4-7/50 treated vs 0/50 controls). At 1000 ppm, an increased (statistical analysis results not reported) incidence of blood-filled cyst(s)/follicle(s) (19/49 treated vs 11/50 controls) was observed. As the magnitudes of increase in incidences were minor at 250 ppm and the lesions were without histological correlate, the effects were not considered adverse at this dose level.

At 1000 ppm, an increased incidence of macroscopic prominent lobulation in the liver in males (15/50 treated vs 1/50 controls) was observed. Increased ($p \leq 0.01$) incidences of diffuse, mainly centrilobular, hepatocellular vacuolation was noted in males (35/50 treated vs 14/50 controls; minimal to moderate) and females (35/50 treated vs 8/50 controls; minimal to marked), and decreased ($p \leq 0.01$) incidences of diffuse hepatocellular vacuolation was observed in males (3/50 treated vs 17/50 controls) and females (5/50 treated vs 33/50 controls). Only minor deceases (↓2-9%; NS) in relative to body liver weights were observed. The biological implication of this redistribution of vacuolation is unclear. In the absence of additional evidence of toxicity in the liver, all these findings were considered of equivocal toxicological significance.

Decreased ($p \leq 0.01$) absolute and relative spleen weights were observed in the 250 and 1000 ppm females (↓38-59%). Hematology and pathology did not corroborate an adverse effect in the spleen. Therefore, the effect on weight was considered equivocal.

In the 1000 ppm females, an increased ($p \leq 0.01$) incidence of minimal to moderate eosinophilic cytoplasmic alteration was noted in gallbladder (13/46 treated vs 3/49 controls). Without corroborating evidence of organ toxicity, the eosinophilic alteration was not considered adverse.

Although the Sponsor cited a decrease in kidney weight in the females, the relative to body kidney weight was only a minor decrease (↓7%; $p \leq 0.01$) and without corroborating pathological evidence of an adverse effect.

The LOAEL is 1000 ppm (equivalent to 142/168 mg/kg/day in males/females). The LOAEL was based on increased incidence of wasted appearance in females; decreased body weights, body weight gains, and food consumption in both sexes; and indications of renal toxicity and hepatotoxicity in males, and stomach and ovarian toxicity in females. The NOAEL is 250 ppm (equivalent to 34/42 mg/kg/day in males/females).

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on the findings detailed above.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

C. **STUDY DEFICIENCIES:** Test compound stability data should be submitted. Otherwise, no deficiency was noted.